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International Study on *Artemia** LVIII. Morphologic differentiation and reproductive isolation of *Artemia* populations from South America

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Abstract. Six *Artemia* populations from coastal and inland sites in Chile, ranging from 20 to 50 degrees latitude south, were compared morphologically. The study included reference samples of *A. franciscana* (San Francisco Bay, California-USA) and *A. persimilis* (Buenos Aires, Argentina). These species are likely to be found in Chile. Samples from Peru (Piura) and Brazil (Macau, Rio Grande do Norte) are two known examples of deliberate introduction of *A. franciscana*. The hypothesis of *A. franciscana* being the dominant species in South America was tested by multivariate morphological analysis based on ten body measurements. In addition, laboratory cross-fertility tests were performed in order to evaluate levels of reproductive isolation among these widely distributed populations.

The analysis showed that *A. franciscana* and *A. persimilis* are morphologically divergent. A number of populations overlap with the San Francisco Bay sample, two are morphologically close to Buenos Aires, whilst others lie morphologically in between. Interpopulation morphological differences along with geographical and, probably, ecological divergence do not reflect reproductive isolation, at least of the pre-mating type. The morphological similarity of two Chilean samples with *A. persimilis* suggests that the distribution of *A. persimilis* should be further investigated. A multi-trait approach for *Artemia* characterization is stressed as a way to obtain better descriptions and interpretations of the biological diversity in the genus.

Key words: *Artemia*, morphological differentiation, reproductive isolation, South America

Introduction

One of the great problems of biology is how a continuous process of evolution can produce the morphological discontinuous groups known as species

* International interdisciplinary study on *Artemia* populations coordinated by the Laboratory of Aquaculture & *Artemia* Reference Center, University of Ghent, Belgium.

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(Coyne, 1992). According to the biological species concept (Mayr, 1969), morphological gaps would be consequence of the reproductive isolation separating members of different species, in concert with selection and genetic drift. Geographical or physical barriers between conspecific populations must exist for reproductive isolation to evolve as a byproduct of the genetic differences accumulated over time (allopatric speciation).

In this paper the brine shrimp *Artemia* is used to compare morphologic differentiation between populations of recently diverged sibling species which are geographically isolated and inhabit variable habitats. *Artemia* is a suitable organism to study the evolutionary processes and the modes of speciation in particular (Browne and Bowen, 1991), i.e. populations exhibit a pronounced tendency for the development of local adaptations, and hence a sequential array of evolutionary stages can often be followed from the population to the species level. This anostracan branchiopod has a striking ability to live and reproduce in hypersaline waters which are mainly located in very dry areas worldwide (Vanhaecke et al., 1987; Triantaphyllidis et al., 1998). These scattered and relatively isolated biotopes offer suitable conditions for maximal inter-population differentiation and ecological isolation (Bowen et al., 1988; Abreu-Grobois, 1987). At least five bisexual and many parthenogenetic species have been described (Browne and Bowen, 1991). Two species are endemic in the New World: the geographically limited (only in Argentina) *A. persimilis* Piccinelli and Prosdocimi and *A. franciscana* Kellogg, the dominant and better studied species in the Americas and the Caribbean. *A. franciscana* is a complex of ecologically-isolated and physiologically-distinct semi species and species (i.e. a super-species) some of which are reproductively isolated in nature (Bowen et al., 1985).

Artemia franciscana is widely distributed in South America (Vanhaecke et al., 1987) either by deliberate inoculation (different sites in Brazil) or through dispersal by waterbirds (Lenz and Browne, 1991). Since introduction or dispersal rapid evolutionary changes seem to have occurred in natural populations of *A. franciscana*, as inferred from the accumulation of novel alleles, apparently as a response to environmental differences (Gajardo et al., 1995). Populations of Macau (Brazil) and Salar de Atacama (Chile) are reported to be changing rapidly, probably due to commercial exploitation and drastic seasonal changes in salinity and temperature (Gajardo and Beardmore, 1993; Gajardo et al., 1995). *A. franciscana*, which as a whole has a highly variable gene pool, displays considerable inter-populational genetic and life history heterogeneity (Abreu-Grobois, 1987). Nevertheless, relatively few systematic attempts have been performed to characterize the pattern of variation on *Artemia* populations in South America. As a result, the biogeography of both *A. franciscana* and *A. persimilis*, the species most likely to be found on

Table 1. Artemia populations, abbreviations and geographical coordinates used in this study.

Country	Locality	Abbreviation	Geographical Coordinates	Type of habitat
Chile	Yape	YAP	20°40′S 70°15′W	Coastal
	Llamara	LLA	21°18′S 69°37′W	Inland
	Salar de Atacama	SAT	23°10′S 68°10′W	Inland
	Los Vilos	LVI	31°58′S 71°25′W	Coastal
	Pichilemu	PCH	34°25′S 72°10′W	Saltwork
	Torres del Paine	TPA	50°29′S 73°45′W	Coastal
USA	San Francisco Bay	SFB	37°28′N 122°30′W	Coastal
Argentina	Buenos Aires	BAI	34°30′S 50°20′W	Coastal
Perú	Piura	PIU	11°14′S 77°35′W	Coastal
Brazil	Macau	MAC	5°00′S 36°40′W	Saltwork
	Unknown	RGR	—	—

this continent, remains to be established (Gajardo et al., 1992; Gajardo and Beardmore, 1993; Amat et al., 1994; Gajardo et al., 1995).

In this work, populations from Chile and other South American countries were morphologically characterized and compared to reference strains of the sibling bisexual species *A. franciscana* (San Francisco Bay, California-USA) and *A. persimilis* (Buenos Aires, Argentina). The assumption that minimal morphological differences should be expected among populations of recently diverged sibling species, together with the idea that *A. franciscana* is the dominant species in the continent were tested by multivariate analysis of adults raised under standardised laboratory conditions. Besides, laboratory cross-fertility tests were performed in order to evaluate levels of reproductive isolation among geographically isolated and ecologically-different (coastal versus inland) populations.

Materials and methods

Samples studied

The following South American *Artemia* populations were studied (see Table 1, Figure 1): Yape, Llamara, Salar de Atacama, Los Vilos, Pichilemu, Torres del Paine from Chile, Piura (Perú), Macau and an unknown locality from Rio Grande do Norte (Brazil). Reference samples of *A. franciscana* (San Francisco Bay-CA, batch 880431) and *A. persimilis* (Buenos Aires, Argentina, batch 534 and 0510/74) were included for comparison. The Torres

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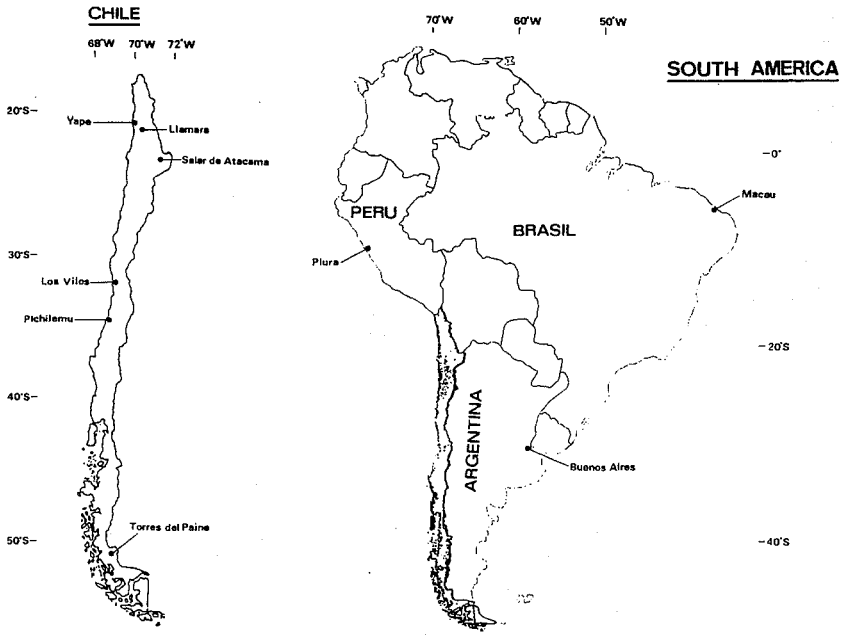

Figure 1. Artemia sites in South America from which populations or cyst used in the study were sampled.

del Paine sample from "Laguna Amarga" (bitter lagoon), located in Torres del Paine National Park (50° to 51° S), Chilean Patagonia, is an interesting and unique new *Artemia* habitat recently described by Campos et al. (1996). This is a semi-arid and humid hydrogeographical zone with oligotrophic lakes. Laguna Amarga owes its saline lake condition to the endorheic basin to which it belongs. It is referred as a coastal site in Table 1 due to its location at the end of the basin and because chloride and sodium are the most abundant ions, though water conditions are particular according to Campos et al. (1996). Climatic conditions of the area are typical (air temperature, wind speed, precipitation and evaporation) of Patagonian conditions (see Table 2 for details).

The Chilean samples were collected as adults in the field and kept under laboratory conditions in aquaria containing 20 L of artificial sea water (35 ppt) prior to analysis. Samples from Buenos Aires (*A. persimilis*), San Francisco Bay (*A. franciscana*), Brazil and Perú were kindly provided as cysts by the Laboratory of Aquaculture & *Artemia* Reference Center, Ghent, Belgium.

Table 2. Basic characteristics of *Artemia* sites in Chile.

Locality	Ecological Characteristics
Playa Yape	A coastal system of small, ephemeral (normally drying out in May), ponds (surface between 4–28 m^2, no more than 60 cm deep). Ponds are relatively close to the shoreline and dry out for several months (November-March). Salinity varies from 52–300 ppt, whilst temperature ranges between 25–33 °C. Small quantity of cysts available. Na and Cl predominantly.
Llamara	A 5 ha saline deposit containing *Artemia* in a main permanent lagoon (1 ha surface, 50 cm depth), surrounded by small ponds. Surface water temperature ranges between 20–25 °C, sharply stratified from June, while salinity fluctuates around 100–170 ppt. pH 7.8–8.0. Na and Cl predominantly, though SO_4 can have some importance in certain months (17 per cent). Cysts not found.
Salar de Atacama	A 3,150 km^2 saline deposit located at a height of 2,400 m in the middle of the Atacama desert. The salar has an underground water level and a system of ponds varying in size from very small to greater ones (more than 200 m wide and 10 m deep). Primary production is very low; the halophilic microalgae *Dunaliella salina* and halophilic bacteria have been detected. Low levels of dissolved oxygen yearround. Ponds are highly seasonal with regard to temperature and salinity. Minimum and maximum water temperature recorded are 6 °C (June) and 32 °C (December), respectively. Salinity ranges 50–280 ppt. Na and Cl predominantly, though SO_4 can reach over 20 per cent. Cysts not available.
Los Vilos	A system of coastal, ephemeral ponds. Only one pond remains with water throughout the year. The amount of water is minimum in April (depth = 15 cm), while it can reach a depth of 1 m (10 m^2 surface) during the rainy season. Salinity range 4–282 ppt. Na and Cl predominantly. No cysts were seen.
Pichilemu	An artisanal saltwork located in an estuarine zone in the South of Chile. *Artemia* was probably introduced by artisanal workers. Great variation in temperature and salinity. Salinity is very low during the rainy season. *Artemia* found at salinities between 30–120 ppt. Na and Cl predominantly. Cysts not found.
Torres del Paine	Population found in Laguna Amarga, Torres del Paine National Park (50° to 51°S), Chilean Patagonia. It is a semi-arid area within an endorheic basin. Average air temperature 7 °C (range: −1–13.8 °C), wind speed very high during spring and summer, average rainfall 639 mm (range: 472–848 mm) most occurring in summer; maximum evaporation in spring-summer, greater (1,200 mm) than precipitation. Water composition: Na, followed by Mg dominant cations, whilst Cl is the most abundant anion followed by bicarbonate-carbonate. Site described by Campos et al. (1996).

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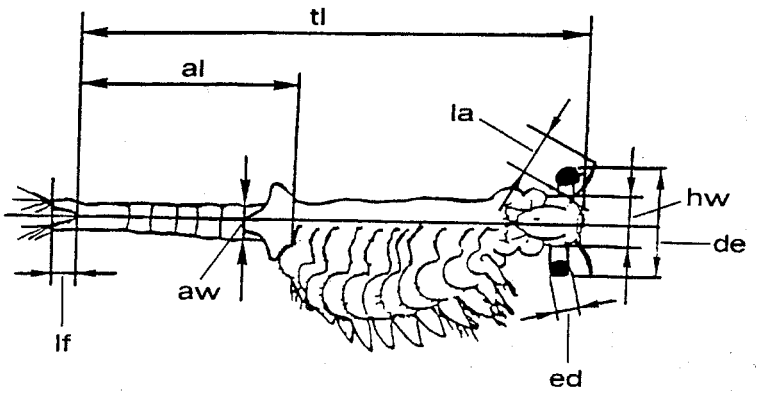

Figure 2. Body measurements in *Artemia*.

Morphological analysis

Body measurements and counts were made on laboratory-reared populations (15 males and females per population) cultured under standardized conditions following the protocol of Amat (1980) with modifications. Animals were fixed in acetic acid (50 per cent), photographed and then measurements were taken directly on photographs as a way to minimize manipulation. The following measurements were obtained (Figure 2): total length (tl), abdominal length (al), abdominal width (aw), distance between compound eyes (de), eye diameter (ed), number of setae per furca (sf), length of furca (lf), length of 1st antenna (la), head width (hw), ratio abdominal length/ total length ($\times$ 100).

Breeding tests

Cross-fertility tests were performed at 65 ppt. Freshly-hatched nauplii were cultured in 2-L aquaria. Prior to sexual maturity randomly taken pairs were transferred into separate vials containing 100 ml of artificial sea water at the desired salinity. On alternative days and just prior to feeding, ovoviviparous offspring were counted and transferred into separate bottles containing 200 ml of brine, where they were maintained until maturity in order to select pairs for the next generation. Oviparous offspring (cysts) were filtered and counted. The observation period lasted 45 days, a time considered sufficient for a major part of the reproductive span to have elapsed (Gajardo and Beardmore, 1989). All observations were conducted at 25 °C and the alga *Dunaliella tertiolecta* was used as food (10^6 cells of microalgae per vial^{-1} per day^{-1}).

Morphological separation of *Artemia* samples from different localities was done by a multivariate discriminant analysis using the computer program

Statgraphics, v. 3.0 (Statistical Graphics Corp., Rockville, USA), with origin of the populations taken as the separation factor. In cross fertility tests, based on F_1, reproductive differences between strains were compared for significance by ANOVA (Sokal and Rohlf, 1991).

Results

Table 1 lists origin, abbreviations, geographical coordinates and type of habitat (coastal, inland) for populations studied, whilst Table 2 summarizes relevant characteristics of *Artemia* sites from which samples were collected in Chile. *Artemia* is found between 20° and 50° latitude South (i.e. more than 4,000 kilometers of distance between extreme locations), in coastal and inland, temporary or permanent ponds varying in ionic composition, salinity and temperature. The climatic conditions of the northern Chilean populations (20° to 35° S) are similar to the climatic conditions described as suitable for *Artemia* by Vanhaecke et al. (1987). In contrast, Torres del Paine, the southernmost Chilean *Artemia* site was found in a semi-arid and humid climate (Table 2).

Morphological variation

Table 3 shows raw body measurements or counts obtained in adults of all studied populations on which the discriminant analysis was based, whilst Table 4 contains the relative contribution of the most relevant discriminant functions. The first four functions give a cumulative separation percentage of 95.55 per cent, though much of the variation is explained by the first and second discriminant functions (68.49 and 11.43 per cent, respectively). Overall discrimination between populations is high as nearly all contributing functions are significant. Figure 3 displays morphological relationships and degrees of separation between populations (centroids provided for each population). Buenos Aires (*A. persimilis*) and San Francisco Bay (*A. franciscana*) are morphologically distinct, whilst a group of populations overlap either with the SFB sample (MAC, RGR, LVI, YAP, PIU) or the Argentinian form (PCH, TPA). Both groups are separated by the first discriminant function which has total length, abdomen total length, number of setae per furca, head width and ratio abdominal/total length (Table 5) as the morphological characteristics contributing most to the observed differences. This function also segregates SAT as a population morphologically intermediate between Buenos Aires and San Francisco Bay. On the other hand, the second discriminant function, with distance between eyes, diameter and length of the first antenna as the relevant

Table 3. Biometrical data for 10 morphological traits in *Artemia* strains from California (USA), Chile, Brazil, Perú and Buenos Aires (Argentina). (n = 30; x and S.D. in brackets).

	(Morphological trait, mm)									
Population	tl	al	aw	de	ed	sf	lf	la	hw	ra*
San Francisco Bay	7.74	2.95	0.69	1.69	0.35	10.95	0.27	0.76	0.94	38.12
	(1.35)	(0.65)	(0.20)	(0.20)	(0.05)	(1.88)	(0.05)	(0.26)	(0.10)	(7.10)
Salar de Atacama	6.97	2.58	0.42	1.31	0.27	7.36	0.21	0.70	0.68	38.10
	(1.14)	(0.73)	(0.90)	(0.16)	(0.05)	(1.71)	(0.06)	(0.21)	(0.12)	(6.15)
Buenos Aires	8.28	3.29	0.40	1.46	0.29	3.10	0.18	0.56	0.76	40.27
	(0.97)	(0.67)	(0.08)	(0.23)	(0.06)	(1.53)	(0.12)	(0.25)	(0.11)	(9.18)
Pichilemu	8.40	4.37	0.42	1.35	0.28	2.88	0.21	0.56	0.77	51.74
	(1.04)	(0.78)	(0.05)	(0.13)	(0.10)	(1.25)	(0.04)	(0.18)	(0.06)	(3.65)
Yape	8.47	3.80	0.53	1.52	0.32	10.16	0.23	0.83	0.86	44.30
	(1.60)	(1.01)	(0.13)	(0.16)	(0.04)	(2.03)	(0.03)	(0.14)	(0.11)	(4.16)
Macau	8.44	3.83	0.62	1.70	0.32	10.16	0.34	0.81	1.02	45.15
	(1.07)	(0.67)	(0.09)	(0.19)	(0.04)	(1.76)	(0.04)	(0.25)	(0.09)	(2.89)
Los Vilos	7.98	3.53	0.54	1.51	0.31	8.70	0.27	0.87	0.81	44.01
	(0.85)	(0.59)	(0.06)	(0.17)	(0.04)	(1.50)	(0.05)	(0.21)	(0.10)	(3.22)
Rio Grande do Norte	7.56	3.18	0.61	1.42	0.29	11.21	0.27	0.78	0.90	41.94
	(0.83)	(0.47)	(0.08)	(0.16)	(0.04)	(1.44)	(0.06)	(0.21)	(0.07)	(2.62)
Piura	5.98	2.49	0.48	1.14	0.24	8.55	0.20	0.73	0.65	41.57
	(0.65)	(0.40)	(0.08)	(0.09)	(0.02)	(1.45)	(0.03)	(0.17)	(0.09)	(2.48)
Torres del Paine	9.37	5.02	0.43	1.46	0.27	1.91	0.06	0.78	0.69	53.37
	(1.29)	(0.88)	(0.04)	(0.24)	(0.10)	(1.40)	(0.05)	(0.31)	(0.09)	(3.55)

*In percent.

Abbreviations: tl, total length; al, abdominal length; aw, abdominal width; de, distance between compound eyes; ed, eye diameter; sf, number of setas per furca; lf, length of furca; la, length of 1st antenna; hw, head width; ra, ratio abdominal length/total length (× 100).

Table 4. Result of the discriminant analysis based on ten morphological traits for *Artemia* populations from California (USA) Chile, Brazil and Perú.

Discriminant Function	Relative Percentage	Canonical Correlation	Significance Level
1	68.49	0.93530	<0.00001
2	11.43	0.73375	0.00001
3	9.95	0.70965	0.00001
4	5.69	0.60586	0.00001
5	2.70	0.46451	0.00001
6	0.89	0.28816	0.00024
7	0.67	0.25343	<0.01
8	0.19	0.13668	n.s.
9	0.00	0.01398	n.s.

n.s.= not significant.

variables, contributes to the separation of PIU (Perú) and Torres del Paine populations (TPA) as well.

Breeding tests

Table 6 shows reproductive data (F_1) (total number of zygotes, cysts and nauplii per female) for nine *Artemia* populations (controls = intrastrain crosses), whereas Table 7 contains pooled data in those cases were reciprocal crosses, including comparisons with BAI and SFB, were not significant. Despite high standard deviations, all inter-population crosses produced relatively abundant offspring. Differences with, at least one of the controls were not significant (ANOVA, $P > 0.05$). Two cases, however, (SFB/PCH and BAI/PIU) gave significantly fewer offspring than the corresponding controls. In addition to the number of offspring produced, it is worth noting that in certain crosses (notably BAI/LLA, SFB/TPA, BAI/YAP, BAI/SAT, BAI/PIU, SFB/PIU) females tended to switch their reproductive mode to cyst production (Figure 4). Reproductive data in those cases where reciprocal crosses were significantly different are presented in Table 8 and Figure 5. Again the total number of zygotes produced is high and so is variability among comparisons. Interestingly, some crosses switched again to cyst production (notably BAI/SFB, BAI/TPA, BAI/LVI, PCH/LVI, PCH/LLA). Cysts proved difficult to hatch by the hydration/dehydration method, and some had a pale-white color in contrast to the usual brownish observed in control groups. Nauplii, on the contrary, reached sexual maturity and abundant F_2 has been obtained in the ongoing tests.

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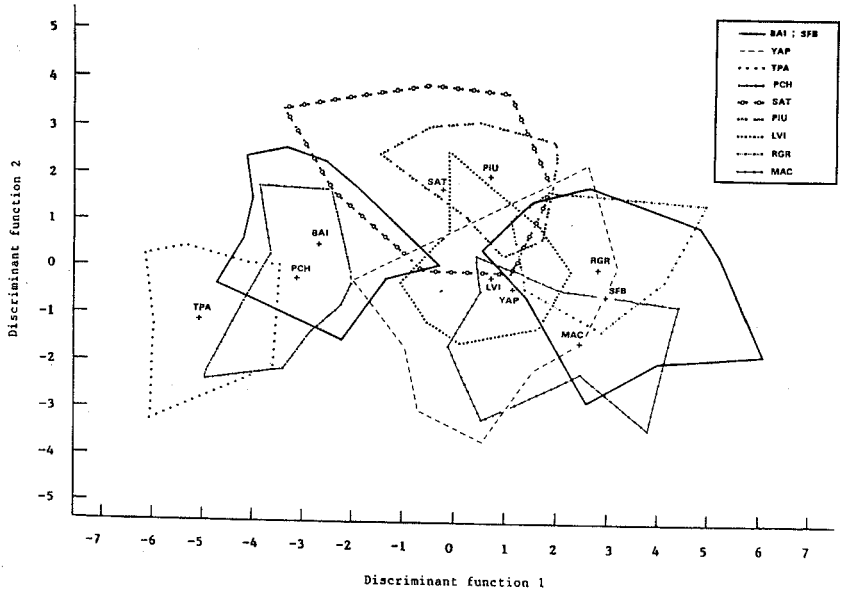

Figure 3. Scatterplot of individual discriminant function scores in *Artemia* samples from California (USA), Chile, Brazil and Perú, including reference strains of *A. franciscana* and *A. persimilis* (+indicates centroids).

Table 5. Standardised coefficients for the first two discriminant functions in ten traits used for the morphological analysis of *Artemia* populations.

Character	Function 1	Function 2
Total length	–0.77	–0.12
Abdominal length	–0.56	–0.32
Abdominal width	0.31	–0.17
Eye diameter	–0.19	–0.45
Distance between compound eyes	–0.04	0.16
Number of setae per furca	0.69	0.04
Length of furca	0.28	0.04
Length of 1st antenna	0.06	–0.18
Head width	–0.65	–0.19
Ratio abdominal length	–0.65	–0.19

Table 6. Reproductive data (x ± S.D.) for nine *Artemia* populations (controls).

Population	*Parental*[1]	*Total zygotes*	*Cyst per female*	*Nauplii per female*
San Francisco Bay	26/29	600.10±421.82	358.20±376.50	241.81±282.37
Buenos Aires	35/35	667.42±198.84	383.48±214.53	284.00±206.40
Llamara	18/18	291.10±199.90	214.27±191.69	77.11± 65.94
Yape	10/11	479.00±344.06	57.10±67.71	421.90±339.24
Salar de Atacama	33/34	486.82±181.63	215.18±190.74	271.58±208.55
Los Vilos	23/23	557.43±258.22	324.61±253.00	232.83±194.03
Pichilemu	17/18	466.44±272.90	295.55±245.24	170.88±205.21
Torres del Paine	9/9	778.33±213.42	727.89±217.76	50.44±110.37
Piura	4/4	328.70±211.56	259.00±110.32	69.75±115.60

[1] number of pairs from which offspring was obtained.

Table 7. Reproductive data (x ± S.D.) for interpopulational crosses between nine *Artemia* samples.

Crosses	Parental	Total zygotes	Cysts per female	Nauplii per female
SFB/LLA	20/20	342.70±046.35	254.05±170.11	88.70±110.72
SFB/YAP	20/21	777.95±304.54	516.10±274.22	261.85±315.69
SFB/PCH	35/41	268.29±134.07	224.06±149.17	44.23±82.03
SFB/SAT	65/67	480.18±250.09	287.34±273.81	192.84±194.37
SFB/PIU	7/8	577.85±131.85	542.14±142.40	35.71±54.39
SFB/TPA	13/13	831.42±394.34	831.42±394.34	0.00±0.00
BAI/LLA	20/20	263.45±230.28	248.45±236.61	15.00±78.00
BAI/YAP	21/24	516.71±402.78	509.19±311.21	7.52±26.22
BAI/SAT	64/67	442.68±266.08	439.84±267.82	2.84±11.96
BAI/PCH	46/47	455.34±215.38	314.26±201.84	141.08±146.54
BAI/PIU	9/9	407.33±320.62	385.11±331.83	22.22±43.54
SAT/LLA	20/20	487.40±246.88	277.20±183.91	210.20±231.04
LVI/LLA	13/14	496.62±293.74	221.23±228.88	275.38±237.09
LVI/YAP	20/20	663.11±196.77	272.95±223.02	390.16±255.19
PCH/YAP	16/17	365.68±228.57	291.64±222.74	53.17±153.72
PCH/SAT	19/19	351.68±216.87	342.84±221.51	8.84±24.58

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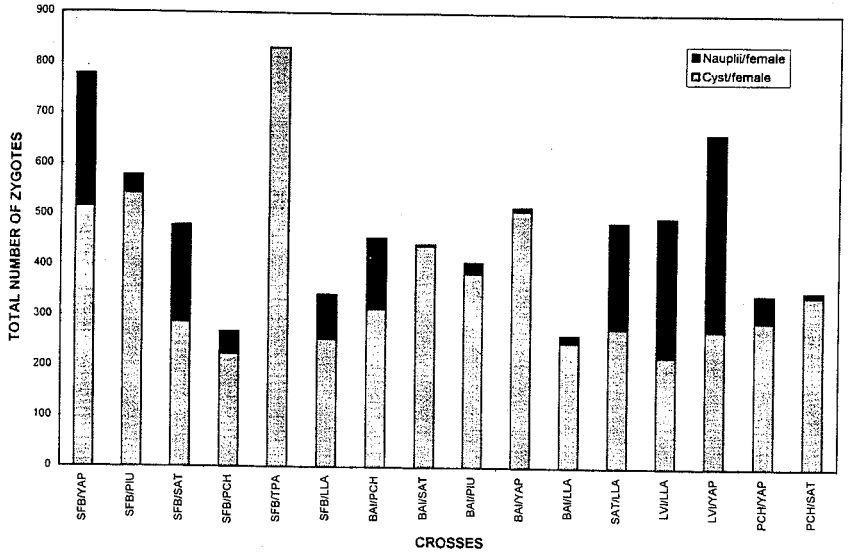

Figure 4. Reproductive performance (N° of cyst/nauplii per female) in interpopulational crosses in *Artemia* (reciprocal crosses pooled).

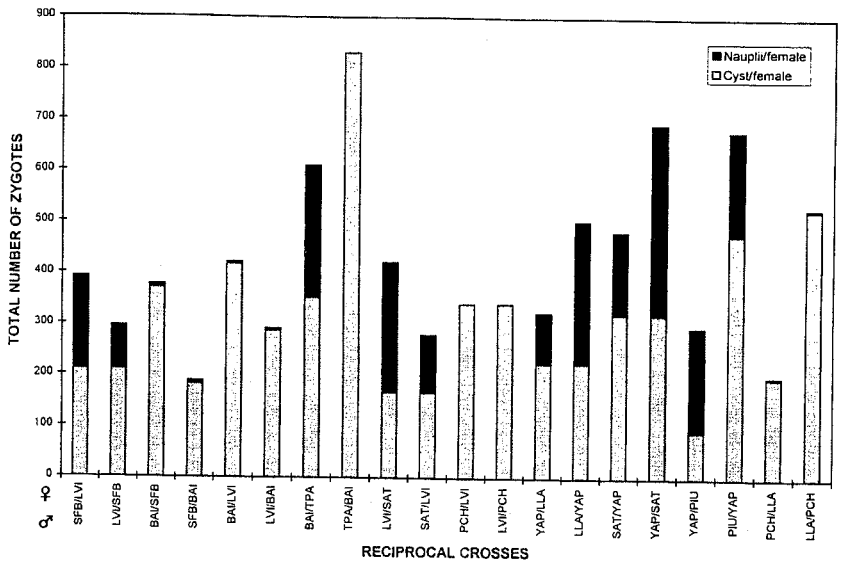

Figure 5. Reproductive performance (N° cyst/nauplii per female) in interpopulational reciprocal crosses in *Artemia*.

Table 8. Reproductive data (x ± S.D.) for interpopulational reciprocal crosses between nine *Artemia* samples.

m/f	*Parental*	*Total zygotes*	*Cyst per female*	*Nauplii per female*
SFB/LVI	25/25	436.32±202.52	211.11±204.27	181.12±184.23
LVI/SFB	20/23	240.95±163.93	**	84.95±107.61
BAI/SFB	9/9	371.33±132.85	371.33±132.85	6.17± 14.46
SFB/BAI	9/9	195.11±131.65	182.77±135.71	**
BAI/LVI	25/25	417.88±148.16	417.88±148.16	4.02±23.03
LVI/BAI	22/23	295.96±216.44	287.56±218.54	**
BAI/TPA	8/8	610.38±169.36	352.00±213.48	258.38±219.20
TPA/BAI	8/8	831.00±130.34	831.00±130.34	0.00±0.00
LVI/SAT	9/9	350.44±161.71	167.11±184.45	254.00±130.06
SAT/LVI	9/9	**	**	112.66±123.31
PCH/LVI	10/10	375.20±089.53	340.55±204.65	0.00±0.00
LVI/PCH	7/10	297.25±027.15	**	0.00±0.00
YAP/LLA	10/10	292.00±013.81	224.75±184.38	98.90±89.33
LLA/YAP	10/10	533.52±286.76	**	276.80±182.99
SAT/YAP	10/10	592.95±183.09	320.16±200.61	162.00±122.46
YAP/SAT	10/10	**	**	372.50±259.12
YAP/PIU	2/2	117.50±037.47	92.50±2.12	202.66±297.83
PIU/YAP	4/7	767.25±087.98	475.75±154.99	**
PCH/LLA	10/10	201.81±063.13	196.54±168.75	2.86±14.71
LLA/PCH	4/4	525.25±093.72	525.25±93.72	**

**Data pooled as reciprocal crosses were not significant for this trait.

Discussion

This study offered an opportunity to expand the knowledge on conditions that promote or constrain morphological differences in the genus *Artemia* and to evaluate under laboratory conditions the degrees of reproductive isolation, i.e.

a key trait for species designation in *Artemia*. Effective overall morphological discrimination based on adult body characteristics was observed between *A. franciscana* (San Francisco Bay, SFB) and *A. persimilis* (Buenos Aires, BAI), while most of the studied populations shared morphological similarity to the San Francisco Bay sample (MAC, RGR, LVI, YAP, PIU) with two exceptions (PCH, TPA) that are closer to the Argentinian population. This finding would lend support to the idea that *A. franciscana* is widely distributed in the continent having reached remote latitudes far south in Chile, whereas *A. persimilis* would be geographically restricted. However, similarity between PCH, TPA (Chile) and BAI (Argentina) questions this distributional hypothesis or, alternatively, cast doubt on the taxonomic reliability of the morphological traits compared.

Many speciation studies have initially dealt with morphological differentiation as the target of natural selection are primarily phenotypes. In *Artemia*, morphological traits have been the basis to describe populations and species, though controversy exits on ways of selecting and using the best traits as well as on their degree of genetic or environmental determination. Since morphological traits can change depending on environmental variation, particularly salinity and/or other culture conditions, authors have sought different analytical procedures to overcome this problem. The simplest and perhaps most effective procedure is to gather analytical or qualitative data for different populations of a species under carefully standardised laboratory conditions (Abreu-Grobois, 1987). Correlations between field and laboratory-raised animals have also been used (Torrentera and Dodson, 1995), but this approach requires careful consideration of intrapond salinity variation which is often observed in natural conditions, particularly with depth. The study of morphological variation and differentiation of *Artemia* populations has also been approached by multivariate analysis of different traits in both cultured and field animals (Hontoria and Amat, 1992; Pilla and Beardmore, 1994; Amat et al., 1994). Such studies have proved particularly useful when there is no prominent morphological distinction between populations, which is often the case in *Artemia*, and because selection is likely to act on many traits simultaneously rather than on a single trait in isolation (Endler, 1986). However, it is advisable to compare multivariate studies to other discriminant characteristics such as allozymes, i.e. phenotypes maybe are the primary target of selective local pressures, but the underlying phenomena are genetics (Pilla and Beardmore, 1994).

In this paper, consistent morphological differences between laboratory-reared populations grown under standardised conditions, suggest an underlying genetic control. Therefore, the comparison of this phenotypic information with any sort of genetic data should provide more biologically useful inter-

pretations. Using electrophoretic procedures Gajardo et al. (1995) confirmed similarity between San Francisco Bay (SFB), Macau (MAC), Rio Grande do Norte (RGR) and Salar de Atacama (SAT), though this latter population would be at the limit (based on genetic distance values) to be considered conspecific. Los Vilos (LVI) and Yape (YAP), as well as Piura (PIU) conform independent groups. On the other hand, cytogenetic information (diploid and chromocentre numbers) (Colihueque and Gajardo, 1996) suggests similarity between MAC, PIU, LVI, YAP and SFB (*A. franciscana*) (all 2n = 42 and > 10 chromocentres), whilst PCH and SAT appear closer to *A. persimilis* (both 2n = 44; no chromocentres and 4.3 chromocentres, respectively). In short, there is good confirmation between morphological and cytogenetic data for MAC, LVI, YAP, PIU and SFB, but this is less evident between morphologic and allozymic data (only SFB, MAC, RGR would be similar). The congruence between morphological and genetic data confirms, as previously suggested, a genetically constrained body organization in *Artemia*. On the other hand, lack of correspondence between the allozymic and cytogenetic data might indicate that these two levels of biological organization exert uncoupled influence upon the phenotype. Hence, a multi-trait approach is to be recommended as a way to provide much better description and interpretation of biological diversity (Moritz and Hillis, 1996). Since many new *Artemia* populations are now being characterized, the use of carefully studied allozymic, cytogenetic and morphological traits that are diagnostic as well as other molecular techniques (see Triantaphyllidis et al., 1997) must be considered. A word of caution, however, should be raised with regard to the relevance of morphological traits. These are often a synergistic combination of size and shape, so reducing differences to a bi-dimensional space of limited biological relevance due to allometry (Bookstein et al., 1985). Hence, much significant differences are likely to be limited to size-independent traits as shown in *Artemia*, where traits such as presence or absence of a structure seem to be of high taxonomic value (Triantaphillidis et al., 1997).

The impact of the environment is relevant to understand the extent of differentiation in *Artemia* populations, often affected by geographical and ecological separation along with climatic and hydrobiological changes which, as a whole, offer a mosaic of environmental conditions that tend to select local forms. Likewise, stochastic processes (genetic drift) are also likely to promote differentiation of local forms in *Artemia* populations which often experience drastic changes in effective population sizes caused by successive cycles of extinction and recolonization, typical of ephemeral or seasonal habitats. It is not surprisingly, therefore, to find high degrees of genetic heterogeneity among the studied populations to the point that some of them could hardly be considered conspecific (see Gajardo et al., 1995).

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Geographical isolation is an effective way to constrain gene flow among natural populations but can be less relevant in *Artemia* as cysts are naturally dispersed over long distances by waterbirds (i.e. flamingos) or wind, counteracting the effect of geographical barriers. Nevertheless, Los Andes Mountains, separating Chile and Argentina, are likely to be an effective barrier to gene exchange particularly in the north and central part of Chile where the highest altitudes are reached. In turn, the Andes tend to disappear towards the Chilean Patagonia, facilitating cyst exchange by birds migration among the two countries. In this geographical scenario, chances are for samples from the north of Chile to share similarity to other *A. franciscana* populations in the continent (assuming a corridor extending along the Western coast of North, Central and South America), whilst the Chilean population from Torres del Paine (TPA, see Figure 1) would be expected to share similarity with Argentinian forms, which diverged independently (Abreu-Grobois, 1987). Results produced in this paper agree with this prediction and are also backed by preliminary allozyme comparison (unpubl. results), though the rapid evolutionary changes, already mentioned, that have promoted rapid divergence of some populations should be beared in mind.

Breeding tests

In *Artemia*, demonstration of reproductive isolation between sexual populations is considered a key for proper characterization of species (Abreu-Grobois, 1987). In fact, gene flow in some geographically or ecologically isolated populations of *A. franciscana* is restricted (Browne and Bowen, 1991). However, isolation as a factor in the acceleration and fixing of developing differentiation seems to be a somewhat more complex phenomenon in *Artemia* since minimal reproductive isolation has been observed in laboratory crosses between geographically wide apart and genetically very distinct populations (Pilla and Beardmore, 1994). Similarly, in this study no indication of reproductive isolation, at least of the pre-mating type, was found between allopatric and genetically distant populations. Nevertheless, while nauplii were abundant and fertile (currently yielding F_3 in experiments in progress), cysts were of a white-pale-transparent type and only very occasionally hatched after being subjected to hydration/dehydration cycles. The fact that morphologically and genetically distant (e.g. BAI/SFB, BAI/ LVI, TPA/BAI) populations tend to switch to the encystment mode of reproduction, while cysts happen to be not viable, could be an indication of reproductive isolation though this requires further testing as recent laboratory experience indicates that more effective methods than the usual hydration/dehydration would be required to deactivate diapause and promote cyst hatching. Nonetheless, Amat et al. (1994) reported hybrid inviability (very few cysts and nauplii

which did not pass 2–3 molts in progenies from parents from divergent populations such as Salar de Atacama (Chile) and Hidalgo from Argentina (*A. persimilis*).

While this study together with others show congruence between morphological and genetic data (allozymes, cytogenetic), there is lack of correspondence between this information and laboratory tests for reproductive isolation. However, this is not unusual as there is evidence that allozymes might play only a minor role in speciation. Instead of changes being produced by many allelic substitutions of small effect at many loci, the more pervasive effects of a smaller number of highly influential genetic alterations (major gene, regulatory genes or chromosomal rearrangements) could be topical for speciation. It has also been suggested that different characters would evolve at a different pace (Avise, 1994). Certain *Artemia* samples have probably evolved very fast for allozymic differences, e.g. Macau and Salar de Atacama (Gajardo et al., 1995), or chromosome characteristics, either through selective pressures or genetic drift (a factor often promoting sudden speciation through the fixation of chromosomic changes). This would be, however, congruent with the idea that New World species would not have developed barriers to gene exchange yet, at least of the pre-mating type (see Abreu-Grobois, 1987). Lack of barriers to laboratory reproductive isolation undermines the universality and reliability of tests for reproductive isolation as the primary criteria for inference of the status of previously uncharacterized populations. However, the relevance of laboratory results should be contrasted in tests better representing natural conditions as, for example, it is known that non-typical water characteristics, or artificial sea water, can affect results.

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